CHLORIDE ION REGULATION AND OXYGEN CONSUMPTION IN THE CRAB OCYPODE ALBICANS (BOSQ)

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There are a number of species of brachyuran crabs which are terrestrial or semi-terrestrial, in contrast to the majority which are strictly aquatic. One of the relatively successful terrestrial crabs of the Atlantic Coast beaches is the "ghost" crab, *Ocypode albicans* (synonyms *O. arenaria,* Say and *O. quadrata,* Fab.). Ocypode burrows in sandy beaches, near or above the high tide level. On the Delaware beaches, where we collected it, the crab is nocturnal and spends the day in its burrow. At night it runs about the beach hunting food, and during this time it may go into the surf briefly. When alarmed, the crab runs for its burrow in preference to the sea. The incoming tide sometimes traps it in its burrow and forms a sand plug in the opening. When the tide recedes, the crab removes this plug and the burrow is useful again. So Ocypode is a crab which successfully lives in a terrestrial habitat, although this habitat borders very closely upon the sea. The anatomical adaptations of this crab have been described by Cowles (1908) and Pearse (1928).

Some brachyurans show a considerable degree of the regulatory ability which results in the maintenance of a steady internal osmotic state regardless of the fluctuations in the external environment. The degree of osmotic regulation of several species of crabs has been described by Jones (1941). He found a direct correlation between the degree to which the regulatory ability was developed and the success the crabs had shown in meeting the conditions of terrestrial life, or life where drying was a constant environmental factor. In his series Uca was the most nearly homoiosmotic. If this correlation can be projected to Ocypode, which belongs to the same family, but is more terrestrial in its habits, Ocypode also should show a high degree of osmotic regulation.

The specific mechanism of water and ion control in Ocypode has not been described. However, it is clear from the literature (Krogh, 1939) that the principle structures concerned in the crustacea are the antennal glands (kidneys) and the gills. The regulatory mechanism certainly operates at these barriers between the internal and external environments; in Ocypode it is not probable that the integument is very permeable to water and electrolytes. The regulation of water and ion movement through a membrane against a gradient is not a passive activity, but requires an output of energy during the process. Schlieper (1929) proposed the theory that homoiosmotic forms respire more rapidly in dilute than in normal sea water, while this is not the case with the poikilosmotic forms, and he suggested that energy expended by the former in resisting osmotic inflow of water is derived from some oxidative mechanism. On this basis it would be expected that any work done by a membrane upon exposure to a changed external environment would be reflected in the oxygen consumption of the animal.

The investigations reported here were concerned with the following aspects of the problem of regulation: first, the degree to which Ocypode carries on chloride ion regulation; second, the role of the antennal gland in chloride ion regulation; and third, a measurement of the oxygen consumption during the early stages of this regulation.

Methods

Crabs were collected at Rehoboth Beach, Delaware, placed in separate containers and brought back to the laboratory within a few hours of capture. They were kept in individual battery jars in which there was enough beach sand to allow burrow digging. This sand was kept moist but not soaking wet. The jars were covered to keep out direct light, and to prevent the escape of the animals. The crabs were fed all the fresh fish they would eat each night and allowed to swim in sea water for about five minutes. The once-a-day feeding and bathing routine paralleled natural conditions as we observed them and permitted us to keep the crabs in good condition for ten days or longer. Crabs had been in the laboratory for at least twenty-four hours before any work was done with them.

The degree of regulation of a single ion, the chloride ion, was studied. It is not presumed that a study of this ion alone gives a complete picture of the shifts in the internal environment. However it may be used as an indicator of the direction and degree of internal regulation.

The chloride ion concentration in the blood from individual crabs was determined. Blood was drawn from the sinus within the proximal joint of one of the legs, using a No. 27 needle fitted on a clean, dry one-quarter cubic centimeter tuberculin syringe. The usual amount of blood drawn at any time was about 100 cubic millimeters. No anticoagulant was used. It was found that quick smooth handling of the blood could effect the transfer from crab to analytical tube before clotting commenced. After the blood sample was drawn, the needle was removed from the syringe, a twenty cubic millimeter Sahli type haemoglobin pipette held to the nozzle of the syringe, and blood measured into the pipette to the calibration mark. The sample was blown into a small test tube, and the pipette rinsed into the test tube with distilled water. Samples were analyzed in duplicate, using a modification of the Volhard method. Silver nitrate was made up to 0.2906 per cent in nitric acid with ferric ammonium sulphate added as an indicator. An excess of this reagent, usually one cubic centimeter, was added to the blood sample in the small test tube. Potassium sulfocyanate solution, made up to one-half the strength of the silver reagent, was used to titrate the excess silver. Reagents were added with the syringe burette described by Scholander (1947).

Urine samples were collected by applying suction to the opercula which close the external openings of the antennal glands of Ocypode. A small vial, capacity approximately two cubic centimeters, was fitted with a rubber stopper through which passed two No. 18 syringe needles. One of these was connected by pressure tubing to a vacuum line. The cuff of the other was removed, and replaced by a short length of soft rubber tubing which could be fitted closely over the operculum. Urine collected in this way was analyzed for chloride ion content by the method used for blood.

The chloride ion present in sea water used was determined by the same method. Sea water taken at the beach from which the crabs were collected was brought back

to the laboratory, filtered and used in the experiments. It contained 480 millimoles of chloride per liter, which conforms to the figure for normal sea water used by Cole (1940). The value is lower than that determined for some open sea samples. This fact may be accounted for considering that Rehoboth Beach, Delaware, is but a few miles south of the mouth of the Delaware River. Distilled water was used to obtain dilutions amounting to 360, 240, and 120 millimoles of chloride per liter. Evaporation in shallow pans under a fan at room temperature resulted in concentrations of 600 and 720 millimoles of chloride per liter. In addition to the sea water concentrations, some crabs were put into distilled water, which is recorded as 0 millimoles of chloride per liter. Others were not put into any of the experimental solutions but remained on damp sand and are recorded as "dry."

The oxygen consumption of the crabs was measured in a respirometer of the direct reading volumetric type, similar in principle to the micro-respirometer of Scholander (1942). The apparatus is shown in Figure 1. The animal chamber

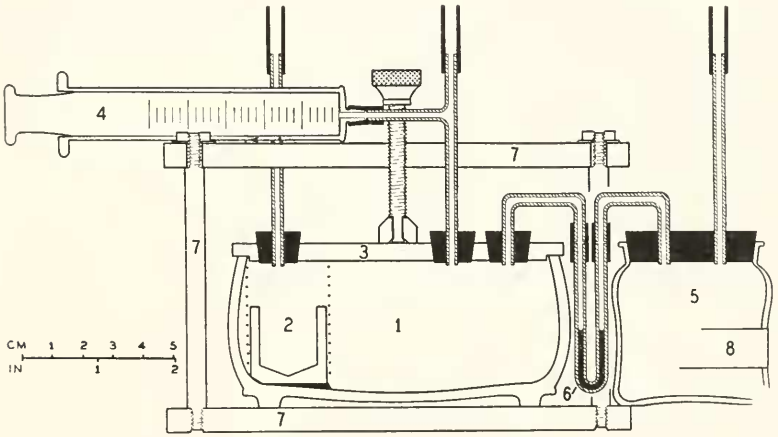

FIGURE 1. A volumetric respirometer (for explanation see text).

(1) was constructed from a finger bowl, the rim of which was ground flat and smooth. An alkali cup (2) made by drilling out a piece of one inch plexiglass rod was fastened to the bottom of the bowl with DeKhotinsky cement. A shield of plastic screen (Lumenite) placed around the cup prevented the animal from getting into the alkali. The lid of the animal chamber (3) was a disc of quarter-inch plexiglass, recessed at the periphery to close on the ground rim of the finger bowl. Three holes were drilled in the disc and fitted with rubber stoppers as shown in the figure. A three-inch length of glass tubing through one of the peripheral stoppers served as a by-pass through which fluids were added to the chamber during an experiment. A "T" tube through the central stopper had a short arm to which a ten cubic centimeter syringe (4) was attached. A small "U" tube passing through the third stopper was connected to the manometer.

The thermobarometer (5) was made from a wide-mouth specimen jar fitted with a two-hole rubber stopper. A by-pass tube through one hole opened the

chamber to the outside air. A small "U" tube connected the thermobarometer to the manometer. The manometer (6) was a tight "U" about two inches long. All glass connections in the unit were made from two millimeter bore glass tubing. Rubber junctions and extensions on the by-pass tube were made from number fourteen rubber catheter. Light weight hose clamps (not shown) were used to close the extensions.

The lid of the animal chamber was closed onto the finger bowl by a stirrup clamp (7). A four by six inch plastic platform was drilled to take two uprights made from quarter inch brass rod. The upper cross member of the clamp was a strip of plastic drilled to pass the threads of the uprights. The center point of this strip was drilled and tapped to take the thread of a thumb screw carrying a knurled knob on one end and a freely turning caster on the end bearing on the lid. The finger bowl rested on the platform. The syringe was fastened to the cross member by heavy rubber bands. The thermobarometer was fastened to one of the uprights with a half inch metal strap (8).

In use the interior of the finger bowl was moistened with water to saturate the atmosphere, and the rim was coated with heavy stopcock grease. Two cubic centimeters of 10 per cent KOH were placed in the alkali cup with a strip of folded filter paper to increase surface for absorption. The interior of the thermobarometer was moistened with water through a by-pass tube. A drop of paraffin oil spread on the plunger made the syringe airtight. The crab was weighed and placed in the finger bowl, which was then set on the platform of the stirrup clamp. The lid was put in position and tightened by means of the thumb screw. The manometer was filled to a height of three-quarters of an inch with 0.1 per cent aqueous tergitol solution and joined to the "U" tubes of the two chambers. A rider made of white plastic sheet was found useful in reading the menisci of the manometer. With all outlets open, the unit was placed in the water bath with the tops of the tubes out of water. The by-pass of the animal chamber was closed. The syringe was charged by attaching an oxygen-filled bladder to the free end of the "T" tube, pulling out the plunger of the syringe, then closing the upper end of the "T" to prevent loss of oxygen from the system. The by-pass of the thermobarometer remained open during the equilibration period of twenty minutes. The syringe was constantly adjusted inward to keep the menisci of the manometer level. At the end of the equilibration period the thermobarometer by-pass was closed, and the initial reading of the syringe recorded. During the course of the experiment oxygen consumed was replaced from the syringe, and the syringe readings gave a direct measure of the oxygen consumption of the animal. The respirometry was done during the summer at a room temperature of 26° C.

The oxygen consumption of the crabs which are described as "dry" in the respiration results was measured in this way for a period of an hour and a half. Regassing the syringe during this time was done without removing the apparatus from the water bath, by attaching a bladder to the "T" tube. Added oxygen was in thermal equilibrium with the water bath. Crabs which were tested for response to a new environment were treated in the following way. The oxygen consumption was determined for thirty minutes, with the crabs in a moist chamber as described. At the end of thirty minutes, the taps were opened, and one hundred cubic centimeters of water of a measured salinity were introduced into the respirometer

chamber through the by-pass tube. This water had been well shaken with air, and had been allowed to equilibrate to the temperature of the water bath. The taps were closed and oxygen consumption of the same crab measured for a full hour. In this way, the sequence of oxygen consumption in air and in environmental fluids of various salinities could be measured.

Blank runs, with the animal chamber moist and with the introduction of water were made following the exact experimental procedure except that no animal was used. The results showed no measurable drift for two hours following equilibration.

Results

Ocypode spends most of its time out of water, either in burrows or running on the sand. The blood chlorides of crabs from sand were determined as a normal value. The mean of sixty-four "dry" determinations was 378 mM Cl/L, with a standard deviation of 24.27. This indicates that two-thirds of the data falls between the values of 354 and 402 mM Cl/L, a spread of 12.7 per cent. The mean is well below the figure for normal sea water, which was 480 mM Cl/L, being much nearer 75 per cent sea water, or 360 mM Cl/L. In view of the spread of the "dry" determinations, it was thought more valuable to compare the reaction of a crab to a new environment with its own "dry" chloride level rather than with a mean of that group or of a control group. The experimental procedure was, then, to determine the chloride of a crab on sand, expose the crab to the experimental sea water, and determine the blood chloride again at specific time intervals. The ratios of the experimental to dry values were calculated. The mean of the ratios for three to six individuals in each environmental situation after one, three, and twenty-four hours are plotted in Figure 2, with the standard deviations in each case represented by a vertical line. One group of crabs remained on sand; the blood chloride was determined for the same time intervals, and treated in the same way. The ratios are recorded in Figure 2 as "dry."

There are no significant changes in the blood chloride levels of individual animals exposed to the various environments for one or three hours. The blood chloride of Ocypode remains steady for a period of twenty-four hours in air, and under the conditions of exposure to sea water diluted to 120 and 360 mM Cl/L and evaporated sea water containing 600 mM Cl/L. There is some difference in the ability of individual crabs to maintain a steady internal chloride level, but the fluctuations observed are not statistically significant. When the crabs are exposed to distilled water or to water containing 720 mM Cl/L for twenty-four hours, the internal chloride does not remain steady, but tends to fall in distilled water and to rise in the high concentration of sea water. Within twenty-four hours there is a significant change in the internal chloride in these extreme ranges although these values do not yet equal those of the external environment. It was found difficult to carry the crabs beyond this time, or to restore them in normal sea water, although they survived three days or longer in all the other environments.

Urine was collected from crabs on sand and from crabs which had been in experimental solutions. An attempt was made to collect urine at one hour, three hour and twenty-four periods, but it was found that the shorter intervals were unsatisfactory. No attempt was made to determine the volume of urine. Ocypode

often ejects the urine when handled, and many samples were lost in this way. Crabs from sand were most often lacking in available urine but it was usually obtained after they had been in any of the solutions for twenty-four hours. The urine chlorides of crabs on sand were determined. The mean of eleven "dry" determinations was 455 mM Cl/L, with a standard deviation of 46. The mean value falls above that for blood, and below that for normal sea water. The results of urine chloride determinations at the twenty-four hour period were treated in the same way as those of the blood chlorides, and are plotted in Figure 3. The mean ratio

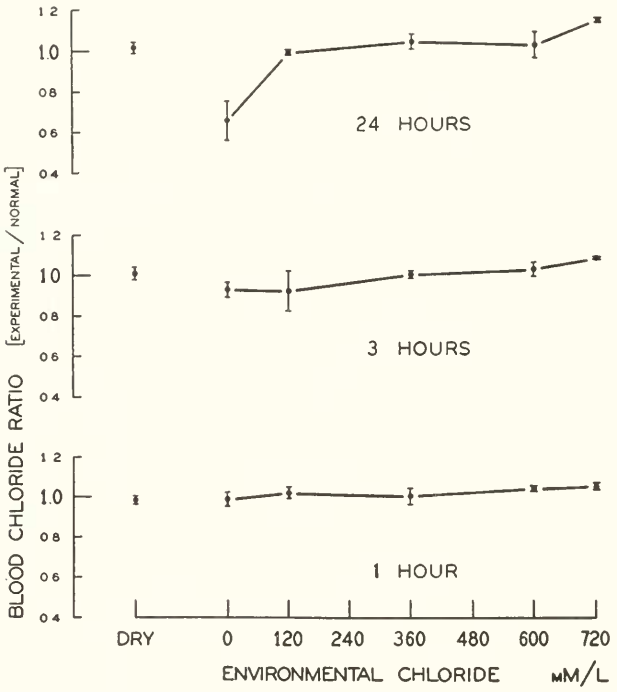

FIGURE 2. Relationship between blood chloride and environmental chloride concentrations.

of urine chloride of three crabs in each environment is plotted, and these points are connected by a solid line. The standard deviation in each case is indicated by the vertical line. The urine chloride ion concentration varies in the same direction as the concentration of the environment, being low in dilute solutions, high in concentrated solutions. There is a wider deviation in the results in the extremely dilute and extremely concentrated environments than there is towards the more nearly isotonic environments.

To determine the effect of the regulatory work on the oxygen consumption, the respiration of crabs from sand, and of crabs in sea water of a range of salinities was measured. The oxygen consumption of six individuals from sand was determined on each of three consecutive days. The mean and the standard deviation were calculated for each of the six individuals, on the basis of data from three con-

secutive daily determinations. The six means and standard deviations so determined were averaged to give the average mean and average standard deviation of six individuals. The mean oxygen consumption was 0.139 cubic centimeters of oxygen per gram fresh weight per hour, with a standard deviation of 0.019. This indicates that about two-thirds of the data fall in the range 0.120 to 0.158 cubic centimeters of oxygen per gram per hour. This treatment gives a more representative picture than would (1) the standard deviation of all eighteen determinations, which would deny that there were six sets of repeat determinations; or (2) the standard deviation of the six means of three determinations each, which would mask the fluctuations within a series of determinations on each crab. It is apparent that the conventional use of a volume of data as the "normal" or control is impossible in the face of such wide deviations. It was decided, therefore, to compare the response

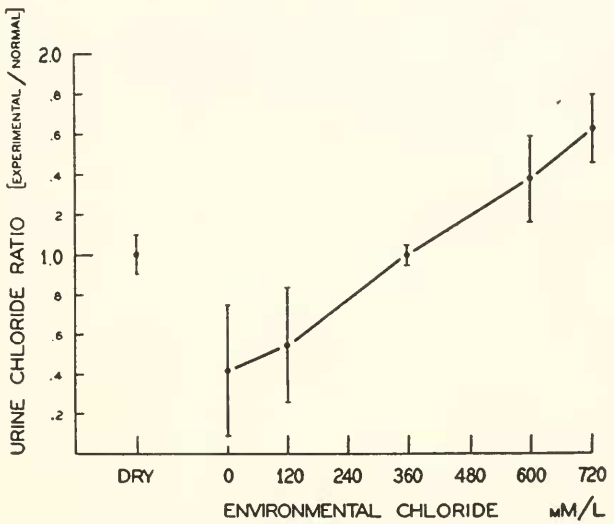

FIGURE 3. Relationship between urine chloride and environmental chloride concentrations after twenty-four hours.

of a single crab to a change in the external environment with the dry oxygen consumption of the same crab. This conformed to the treatment of the chloride data.

In the respiration measurements, the oxygen consumption of the crabs was determined the first thirty minutes with no standing water in the moist chamber, hence the animals could be termed "dry". This thirty minute period is designated "A". The oxygen consumption was determined for one hour after the experimental sea water was added to the respirometer. The first thirty minutes after the addition is designated "B", and the second thirty minutes "C". It could be predicted that if the oxygen consumption rate did not change, but a steady rate was maintained during the entire ninety minutes, period "B" would equal "A", and periods "B" plus "C" would equal twice "A". A graph of this steady state would be a straight line.

TABLE I

Environment in mM Cl/L	"B"/"A"	"B" + "C"/"A"
0	1.50	2.53
120	1.54	2.59
240	1.58	2.73
360	1.15	2.17
480	1.41	2.69
600	1.38	2.57
720	1.35	2.61
"Dry" (normal) predicted ratio	0.95 1.00	1.84 2.00

The data on oxygen consumption were calculated as ratios of the period "B" to period "A", and of period "B" plus "C" to period "A". Six individuals were tested on three consecutive days for each of the environmental situations, and the data calculated as outlined above for the normal rate of oxygen consumption. Table I presents the mean of the ratios so obtained. In Figure 4 the data are presented graphically, with the mean of six individuals plotted as the central point, and the mean standard deviation indicated by a vertical line for each set of determinations.

The rate of oxygen consumption was consistently higher in water, regardless of the concentration, than under the dry circumstance. This cannot be attributed

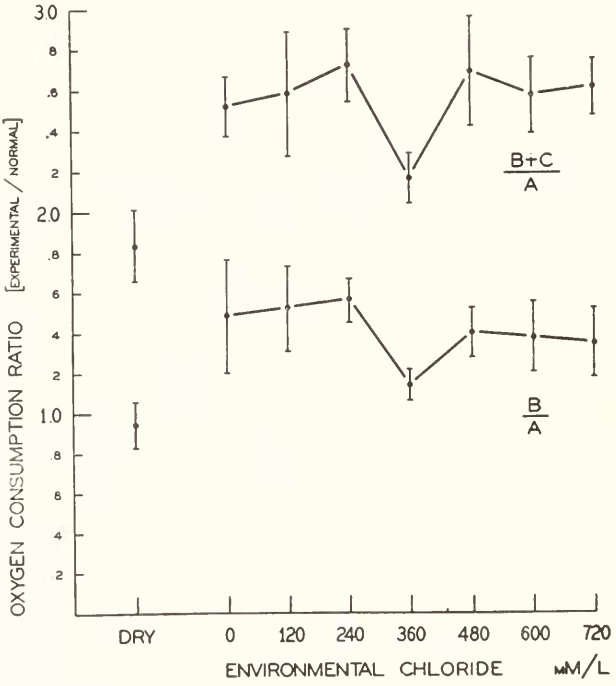

FIGURE 4. Oxygen consumption related to environmental chloride.

to diffusion of gas into the introduced water or oxygen consumption by organisms in the water. Check runs complete except for the crab failed consistently to give discernible evidence of any but a steady state of the respirometer. The rate of oxygen consumption in water containing 360 millimoles of chloride per liter was significantly less than the rate in any other salinity. This concentration most closely approximated the normal internal chloride concentration of the crab, which was determined as 378 millimoles of chloride per liter. In concentrations of sea water greater than or less than this nearly isotonic concentration, the rate of oxygen consumption increased significantly. The increase in dilute solutions was greater than in hypertonic solutions. There was no evidence of progressive increase as the extremes of salinities were approached. There was, however, a wider fluctuation in individual results, particularly in the dilute series.

Discussion

Ocypode maintains a steady chloride ion concentration in the blood in air and against a wide range of external chloride concentrations. The mean blood concentration is 378 millimoles of chloride per liter, and is held steady against external concentrations ranging from 120 to 600 millimoles of chloride per liter. When the crab is exposed to external concentrations of 0 and 720 millimoles of chloride per liter the steady state is not maintained and a drift towards the lower or higher value is observed. At the end of twenty-four hours in these two extremes, the chloride ion concentration in the blood falls as much as 40 per cent or gains as much as 15 per cent over the normal level. No observations were obtainable on crabs which had been exposed to the environmental extremes for several days. It was very difficult to keep them alive beyond the twenty-four hour period, especially in distilled water. The decreasing viability probably is not due to the shift in chloride ion concentration alone, but is indicative of a breakdown in the mechanisms regulating water and ion transport.

The ability of Ocypode to regulate the chloride ion concentration of the blood is in agreement with the observed ability of the more terrestrial crabs, Uca, in particular, to approach a homoiosmotic condition. Jones (1941) has shown that Uca regulates against both hypotonic and hypertonic environments. The fact that the mean value of blood chloride for Ocypode is well below the figure for sea water, which was 480 millimoles of chloride per liter at the site where the crabs were taken, indicates that the crabs which made the transition from sea to land possess the mechanism fundamental also to those which move from sea to brackish water. According to the hypothesis proposed by Beadle and Cragg (1940), a contributing factor to successful transition from sea to brackish or fresh water is the maintenance of a chloride ion concentration in the blood somewhat lower than that of sea water, hence, presumably easier for the animal to maintain against the brackish water gradient. Other members of the family Ocypodidae, Uca for example, invade brackish water as well as spending much time in air. The lower internal chloride concentration of Ocypode is obviously easier to maintain in the terrestrial habitat wherein food and infrequent contact with sea water are the only sources of chloride ions.

The maintenance of a steady internal chloride ion concentration is accomplished by regulation of water intake and loss, and salt intake and loss. The data we have

collected on urine chloride concentrations indicate that the antennal gland of Ocypode functions in chloride ion control. If the chloride ion content of blood and urine from crabs on sand, the "dry" or "normal" values, are employed with the ratios shown in Figures 2 and 3, and the representative levels of blood and urine chloride under the imposed environmental conditions are calculated, a graph such as Figure 5 may be made. Calculated levels for urine chloride are indicated by a solid line,

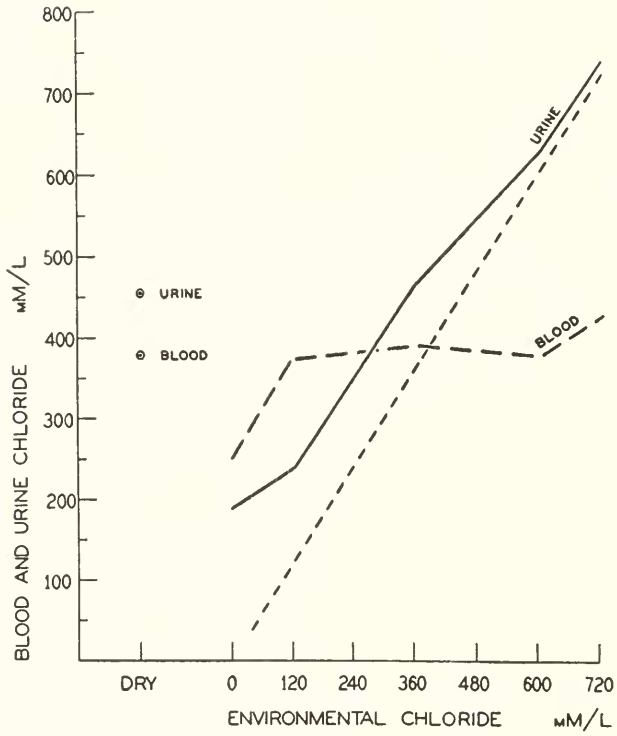

FIGURE 5. Calculated blood and urine chloride concentrations related to environmental chloride.

for blood by a broken line, and for the environmental fluid by a dotted line. The line representing the environmental fluid crosses the line for blood chloride at the isotonic level, about 380 millimoles of chloride per liter. In the situation of the chloride ion concentration in the external environment being equal to that in the internal environment, no accumulation of chloride in the urine would be expected. However, as the data indicate, chloride ion is present in the urine in excess at the isotonic point. This is probably due to the re-absorption of water by the antennal gland. Supporting evidence for this conclusion may be drawn from two observations: first, the urine chloride ion content when the crab is in a medium containing 360 mM Cl/L is not significantly different from the urine chloride concentration of crabs on sand. Water saving by re-absorption is important for a terrestrial animal, and it would appear to be the activity reflected here. Second, the oxygen consumption of crabs in a medium containing 360 mM Cl/L is the lowest of all the rates of

crabs in fluid environments, indicating a minimum of regulatory work being done.

The chloride ion concentration in the urine varies in the same direction as that in the environment. However, the urine chloride concentration is equal to the blood concentration only when the crab is in an envionment containing about 270 mM Cl/L. In sea water containing more chloride than this value, the urine tends to contain more chloride ion than the blood; in more dilute media the concentration of chloride ion in the urine is less than in the blood. Here is indication that the antennal gland is active in the control of the chloride ion concentration in the internal medium, either actively secreting chloride or re-absorbing water, over a considerable range of external environmental salinity. This is an expected activity when the chloride ion concentration in the environment is higher than that in the blood. Further examination of the data shows that in very dilute solutions the antennal gland wastes chloride, producing a urine that has a high chloride ion content as compared with the external environment. The fact that a high urine chloride loss persists in distilled water, despite the fact that the blood chloride level is falling, may be interpreted to mean that the antennal gland does not have a critical threshold for the chloride ion.

From Figure 5 it may be seen that at the 270 mM Cl/L level in the external environment, blood and urine chloride concentrations are equal. Animals in this range of environmental chloride survived for several days and maintained a normal and steady blood chloride level. It is clear that at this point proportionately more chloride than water must be obtained from the external environment. The fact that the blood chloride level is maintained steady in hypotonic environments in spite of an overactive excretion of chloride by the antennal gland is further evidence that some chloride-absorbing mechanism is at work. It is assumed that the mechanism here is selective absorption of chloride ion by the gills. Webb (1940) has shown that the gills of Carcinus do actively absorb chloride ion from the external medium. He has further shown that there seems to be no reason to assume an irreciprocal permeability of the gut and integument. It might be pointed out that the integument of Ocypode does not appear to permit evaporation of water when the animal is on land, and it is assumed that it would not permit great exchanges of salt and water when the crab was immersed. We have not yet completed studies as to the possibility of specific tissues being reservoirs of internal salts and water.

It is seen from the data on oxygen consumption of crabs from sand and from environments of different salinities that work is being done to maintain the internal chloride ion concentration, and, it may be projected, the internal osmotic state. The membranes involved, gill and antennal gland, although affording ample surface for the exchange of materials, do not make up a large part of the mass of the animal. In order to determine the metabolic reaction to changing environments it is necessary to use an intact animal, and to measure a change dependent on the activity of a very small per cent of the weight of the animal. It was felt that with the combination of measuring a small overall change and the inherent variation between individuals, statistical treatment of the data would prevent overlooking pertinent relationships.

In order to relate the data obtained on oxygen consumption to the observed regulation of the internal chloride ion concentration, the ratios of the first experimental period to the control period ("B"/"A") are represented in Figure 6 in block

form. If the oxygen consumption in air (solid segment) is assumed to represent the normal or control value, and if this amount is then removed from the values for oxygen consumption in water, the increment in water must be due to work, either of a mechanical or osmotic nature. The increment is least, about 21 per cent, in an external environment of 360 mM Cl/L. From Figure 5 it is seen that at this concentration the blood and external chloride concentrations are nearly equal. As suggested earlier, at this environmental level, the gill is probably doing little or no osmotic work, since no gradient exists between internal and external environments. On the basis of the urine chloride data, the antennal gland work at this level is no more than is being done by the "dry" crab on sand. Therefore, most, if not all of the increment in oxygen consumption at 360 mM Cl/L is due to

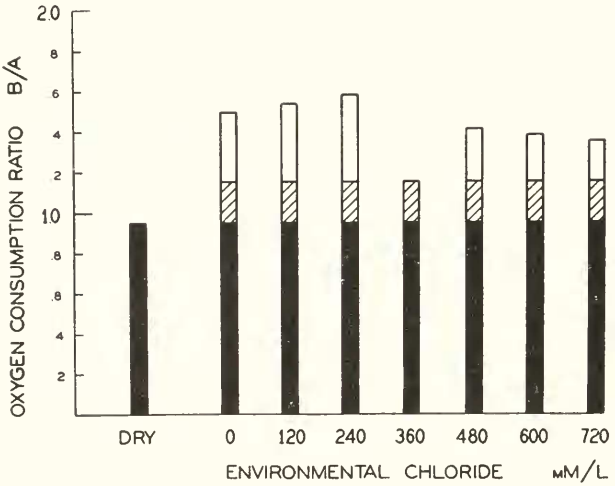

FIGURE 6. Oxygen consumption related to environmental chloride to show partition of work between air and water ventilation and between water with and without chloride gradient.

mechanical or muscular activity. In view of the greater mass and viscosity of water it is reasonable to assume that water bailing requires more energy than air ventilation. Ocypode has reduced gills and branchial tufts according to Pearse (1928). In air the diffusion of oxygen through these respiratory surfaces is sufficient for the requirements of the animal, probably with no more accessory ventilation than is accomplished during leg movements. In the respirometers the crabs showed a definite thigmotactic response and were quiet during the time of the experiment. When Ocypode is placed in water, a scaphognathite attached to the second maxilla sets up a rapid beat which serves to force water from the posterior branchial chamber opening between the bases of the third and fourth legs through the branchial chamber and out at the mouth region. Two "gill rakers" attached to the first and third maxillipeds also beat rapidly when the crab is in water. This constant muscular activity which is not present in crabs in air may account for the increase in oxygen consumption in environmental fluid containing 360 mM Cl/L.

A possibility which merits further investigation is that some of the increase noted when the animals were placed in water may be due to the payment of an oxygen

debt incurred as a result of drying of a significant portion of the respiratory surface, or as a result of inadequate ventilation in air when the animal is quiet. In an environment containing 360 mM Cl/L, the ratio of respiration in the first experimental period to the control period is 21 per cent higher than the same ratio of crabs "dry" throughout the experiment. The ratio for the second experimental period is only 14 per cent higher than the same corresponding ratios for "drys." Measurement of the respiration was not continued past this point. Not enough is known of the respiration pattern of Ocypode to make a definite statement concerning the possibility that these increments indicate payment of oxygen debt. However, two observations may be considered here: (1) crabs on moist sand ten days are just as active and maintain the activity as well as crabs exposed to periodic wetting, which would indicate no cumulative oxygen debt; and (2) the pattern of decrease in the second period does not change with increasing time of exposure to air. The possibility of early saturation of the oxygen debt capacity complicates this latter observation. It may be speculated that if there is an oxygen debt present in Ocypode in air, this represents a phase of terrestrial adaptation which has not yet become perfected in this species.

The increase in oxygen consumption in 360 mM Cl/L as compared with that in air is represented in Figure 6 by the cross hatched segment. If this portion, plus that corresponding to the respiration in air, is then removed from the total block for each environment, the remaining clear segment may be assumed to represent the osmotic work done in maintaining a steady internal environment against a gradient. In hypotonic solutions the increase attributable to this regulatory work is of the order of 34 per cent, decreasing slightly in the more dilute environments. This increase represents the sum of the work of two regulating mechanisms. The gill membrane is working to absorb chloride ion from the environment, and is allowing water to pass into the crab, while the antennal gland is bailing water out again. In the more dilute environments the antennal gland appears to be inefficient. In Figure 5 it is seen that the amount of chloride excreted is in excess of what would be predicted on a straight clearance basis, which would indicate that the antennal gland is failing to re-absorb chloride ion or is not bailing the water necessary to maintain the balance. Webb (1940) has suggested that in very dilute solutions the gill of Carcinus becomes less permeable to water. The data presented here seem to be in accord with this suggestion. The gill continues to absorb chloride ion from the environment, while allowing less water in proportion to pass the barrier; the antennal gland then clears less water, with less energy expenditure than would be expected. The two mechanisms are able to maintain an internal chloride ion level in solutions containing 120 millimoles of chloride per liter or more. No points intermediate between this dilution and 0 were tested, so that the exact limit to which the regulation is maintained is not known.

In solutions containing more than 360 mM Cl/L there is an increase in the rate of respiration of the order of 20 per cent. In hypertonic solutions the gill ceases the selective absorption of chloride ion from the environment, and as far as we know, has no chloride-secreting mechanism. However, chloride ion and water pass through the gill membrane in accordance with the Donnan equilibrium. The antennal gland is the chief mechanism of regulation in hypertonic environments, excreting chloride ion or saving water, or both. The antennal gland working alone

does not require as much oxygen as gill and antennal gland together do in hypotonic solutions. In environments of high concentration, 720 mM Cl/L, the ability of the antennal gland reaches a limit and cannot keep up with the incoming chloride ion. Hence, the blood chloride ion level rises. The upper limit on antennal gland ability is also shown by the fact that the oxygen consumption does not progressively increase with increase in environmental chloride. The slightly lower oxygen consumption in the greatest concentration may be the result of metabolic depression caused by the concentration of ions other than chloride.

With the hypothesis of Schlieper (1929) in mind, inspection of the oxygen consumption rate across the spectrum of osmotic insult would indicate the point at which the external and internal environments approached isotonicity. The elevation of the oxygen consumption rates on either side of this isotonic point may be accounted for, as indicated above, by the energy output of gill and antennal gland, each in a twofold role of chloride ion capture and clearance.

Further examination of the respiration and urine chloride data indicates that the deviation from the mean is much wider at the environmental extremes, than towards the isotonic level. It may be postulated that here is shown a range of response of single factor magnitude which might form a basis for selection. In the past, the antennal gland function, especially on the hypertonic side and in air, may have been an important factor in the selection of crabs as they passed from water to the terrestrial habitat.

Summary

1. *Ocypode albicans,* a brachyuran crab with a terrestrial habitat, is able to regulate the chloride ion content of the blood in the dry habitat and in sea water of salinities ranging from 120 to 600 millimoles of chloride per liter. The mean value of the chloride ion concentration in the blood is 378 millimoles of chloride per liter. This value is lower than that of sea water at the site the crabs were collected (480). It is suggested that the lower concentration of chloride is easier for the terrestrial animal to maintain in an environment where chloride is available only from food or from occasional contact with the sea.

2. In sea water containing less than 120, or more than 600 millimoles of chloride per liter, the internal concentration is not maintained, but tends to fall in dilute solutions, rise in the more concentrated ones. This internal shift is significant in a period of twenty-four hours, and proves fatal if continued for an additional twenty-four hours. The antennal gland functions in the regulation of the internal chloride ion concentration. It re-absorbs water when the crab is in air and in hypertonic solutions. It excretes water when the crab is in hypotonic solutions, and may excrete chloride also. The evidence points to another organ functioning in the reverse way; this is assumed to be the gill membrane.

3. The oxygen consumption of Ocypode in air is of the order of 0.139 cubic centimeters of oxygen per gram fresh weight per hour. In water the crab uses a gill bailer and gill rakers constantly, and the respiration rate is elevated. The increase is least when the crab is in an environment in which the chloride ion concentration equals that of the blood. In hypotonic and hypertonic environments the oxygen consumption is further increased. This is caused by the increased activity of the ion-regulating membranes of the antennal glands and gills.

4. Comparison of individual responses to the different environments indicates a difference in individual ability to meet the stress. The differences are more widely marked in environments most different from the internal medium. It is suggested that this is a measure of the variation of a physiological mechanism which was probably important in the evolution of the crab from the aquatic to the terrestrial habitat.

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